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Article

Molecular Detection and Immunological Characterization of *Leishmania donovani* in patients in Wasit province, Iraq

Baraa Abdulsalam Hraija[*1]

1. Department of Microbiology, College of Medicine, University of Wasit, Wasit, Iraq
Correspondence: babdulsalam@uowasit.edu.iq

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Abstract: Visceral leishmaniasis is a protozoan parasitic disease of vector-borne transmission with a notable increasing in global incidence and spreading to novel geographic areas. In Iraq, molecular data concerned visceral leishmaniasis remain limited and need to support. Molecular surveying of *Leishmania donovani* in clinically asymptomatic adults, sequencing and phylogenetic analysis of study isolates, with estimation the levels of some immune markers including IL-1β, IL-10, IFN-γ, and TNF-α. An overall 285 rural adults of 10-70 years age-old and both sexes were selected to sampling of venous blood that divided equally into EDTA-anticoagulant tube for molecular testing by conventional PCR, and free-anticoagulant tube that centrifuged to obtain sera for immunology by quantitative ELISA. The positive *L. donovani* isolates were sequenced and analysed phylogenetically using the MEGA-11 software and NCBI-Viewer. Molecular findings of PCR assay reported that 4.21% of study population was positively infected with *L. donovani*. In comparison with the global NCBI-BLAST *L. donovani* isolates / strains, phylogenetic tree analysis, MSA and homology sequence identity for study *L. donovani* isolates demonstrated the presence of significant identity with the Brazilian *L. donovani* isolate (GenBank ID: ON934698.1) at a similarity ranged from 98.50-99.94% and mutation / changes ranged from 0.0003-0.001%. Immunologically, the positively infected individuals express a significant reduced IL-1β, but elevated serum levels of IL-10, IFN-γ, and TNF-α. This represents the first molecular study in Wasit province, and the first phylogenetic one in Iraq demonstrating that there was a close-relationship between the study *L. donovani* isolates and the global NCBI-GenBank isolates, and estimating levels of some immune markers in infected cases. Author suggests the great necessity of additional molecular phylogenetic and immune studies in cases of leishmaniasis.

Keywords: Interleukin, Kala-Azar, NCBI, Qualitative PCR, Sequences and Phylogenetic Analysis, Visceral Leishmaniasis

Introduction

Leishmania donovani is a haematoflagellate, kinetoplastid, intracellular, protozoan parasite which belongs to the family Trypanosomatidae under the Euglenozoa phylum (Tao and Jia, 2024). The parasite is responsible transmitted to humans via the bite of infected female of phlebotomine sand flies

(Diptera: Psychodidae) and invade the macrophages to proliferate within phagolysosome (Al-Joary and Al-Hamdani, 2024; Tom et al., 2024). This leading to development a systemic disease known as visceral leishmaniasis or Kala-azar that clinically ranged from asymptomatic infection to severe life-threating form based on the host immune response (Abbas et al., 2019; Volpedo et al., 2021).

The intricate interplay between the host immune system and parasitic pathogens is crucial for developing effective control strategies and therapeutic interventions (Yasmin et al., 2022). Interleukins, as key mediators of immune responses, play a fundamental activity in orchestrating host defense mechanisms against parasitic challenges throughout secretion of specific proteins to regulate the differentiation, proliferation and activation of various immune cells (Wen et al., 2021; Obeagu, 2024). Dysregulation of pro-inflammatory interleukins such as IL-1 can contribute to severe pathology, while conversely; anti-inflammatory interleukins like IL-10 can prevent excessive inflammation and tissue damage, yet their overexpression compromises effective parasite elimination and promote chronic infections (Saha et al., 2022; Al-Qahtani et al., 2024).

Geographically, leishmaniasis is endemic in many parts of approximately 90 countries, primarily in the tropics and subtropics including Iraq (Al-Hayali and Al-Kattan, 2021). This warrants a critical evaluation of factors such as under-reporting asymptomatic infections and emergence of drug resistance which collectively contribute to the persistent burden of disease (Perez et al., 2024). Consequently, a significant portion of increasing the global incidence of disease, estimated to be the ninth largest among individual infectious diseases, remains markedly unaddressed due to complex epidemiology, insufficient current incidence data, and limited diagnostic tools (Abbasi, 2025). Diagnosis relies usually on a combination of clinical suspicion, parasitological confirmation, and immunological tests; however, each presenting unique challenges and frequently suffer from low sensitivity and specificity, especially in endemic areas with high rates of co-infections and varied clinical presentations, often leading to delayed or inaccurate diagnosis (Kumar et al., 2020; Reimão et al., 2020; Gow et al., 2022). Currently, advanced molecular diagnostic techniques have offered high sensitivity, specificity, and rapid detection capabilities, making them suitable for point-of-care testing in resource-limited settings (Mehrotra et al., 2025). In Iraq, few available studies have been conducted to identify the incidence rate of visceral leishmaniasis using one or multiple methods including rK-30 strip tests or immunochromatography test (ICT), bone marrow or spleen aspiration, microscopy, indirect fluorescent antibody technique (IFAT), and molecular assays (Rahi et al., 2013; Al-Ardi, 2022; Dakeel and Mohsein, 2024; Jaffar et al., 2024). Therefore, this study aims to molecular surveying of visceral leishmaniasis caused by *L. donovani* in clinically asymptomatic adults, sequencing and phylogenetic analysis of study isolates, with estimation levels of immune markers including IL-1◎ and IL-10.

Materials and Methods

Ethical approval

The Scientific Committee in the College of Medicine (University of Wasit) was licensed the current study.

Samples

An overall 285 rural adults of 10-70 years age-old and both sexes who admitted to some private laboratories in Wasit province (Iraq) during April (2025) were selected and subjected to draining 5ml of venous blood. Then, each blood sample was divided equally into two tubes; EDTA-anticoagulant tube to be used for molecular testing, and free-anticoagulant tube that centrifuged at 5000rpm for 5 minutes and the obtained sera was saved into 1.5ml Eppendorf tube to be applied for immunology. All EDTA-whole blood and serum-Eppendorf tubes were saved frozen (-40°C) until be tested.

Molecular testing

DNAs were extracted from the EDTA-whole blood samples throughout the gSYNCTM DNA Extraction Kit (Geneaid, Taiwan), and evaluated spectrophotometerically using the Nanodrop. Then, AccuPower® PCR PreMix Kit (Bioneer, Korea) was served to preparation the MasterMix tubes with

utilization of extracted DNAs and the designated primers (F: 5'-CGC ACC GCC TAT ACA AAA GC-'3 and R: 5'-ATC CTG GTC ACA GCC TCT CT-'3) for this study based on NCBI-GenBank *L. donovani* isolate [ID: ON796536.1 (https://www.ncbi.nlm.nih.gov/nuccore/ON796536.1)]. For PCR reaction, the tubes of MasterMix were transferred to the Thermal Cycler system, and then subjected to electrophoresis of agarose-gel (1.5%) at 80Am and 100Volt for 90 minutes, and the bands of positive samples were visualized under the ultraviolet transilluminator at 618bp

Phylogeny

The DNAs of positive samples were sequenced, and submitted in NCBI-GenBank database and analysed phylogenetically by the MEGA-11 software and NCBI-Viewer to indicate sequence identity between the study and the NCBI-BLAST *L. donovani* isolates.

Immune markers

Initially, the serum samples were thawed in water bath at 37°C, and the components of IL-1◎ (Cat. No: SL0984Hu), IL-10 (Cat. No: SL0967Hu), IFN-◎ (Cat. No: SL0960Hu), and TNF-◎ (Cat. No: SL1761Hu) kits were prepared at room temperature. Following the manufacturer instruction of each kit (SunLong Biotech, China) separately, the sera and Standard solution of each kit were processed step by step, and the ODs were measured at an absorbance of 450 nm using the ELISA Reader system. For calculation the concentrations of each immune marker, ODs and their consequent concentrations of the Standards in addition to ODs of all samples were plotted on the log scales of standard curve.

Statistical analysis

The t-test in the GraphPad Prism Software was utilized to identify variation among positively and negatively infected individuals at a significance of $p<0.0001$ (****), $p<0.001$ (***), $p<0.01$ (**), and $p<0.05$ (*). Values were represented in the current study as either number (percentage) or mean ± standard error (Ajaj et al., 2021; Gharban et al., 2024).

Results

Molecular phylogeny

Targeting the small subunit rRNA gene, molecular findings of conventional PCR assay revealed that 4.21% (12/285) of study population were positively infected with *L. donovani* isolates (Figure 1). Sequencing data of all positive study *L. donovani* isolates were submitted in the NCBI-GenBank database under the names (access numbers) of Iraq-HABR1 (PQ357484.1), Iraq-HABR2 (PQ357485.1), Iraq-HABR3 (PQ357486.1), Iraq-HABR4 (PQ357487.1), Iraq-HABR5 (PQ357488.1), Iraq-HABR6 (PQ357489.1), Iraq-HABR7 (PQ357490.1), Iraq-HABR8 (PQ357491.1), Iraq-HABR9 (PQ357492.1), Iraq-HABR10 (PQ357493.1), Iraq-HABR11 (PQ357494.1), and Iraq-HABR12 (PQ357495.1). In comparison with the global NCBI-BLAST *L. donovani* isolates / strains, phylogenetic tree analysis, MSA and homology sequence identity for study *L. donovani* isolates demonstrated the presence of significant identity with the Brazilian *L. donovani* isolate (GenBank ID: ON934698.1) at a similarity (*) ranged from 98.50-99.94% and mutation / changes ranged from 0.0003-0.001%, see Table 1, Figures 2-5.

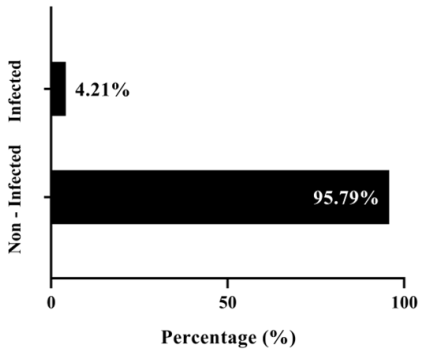
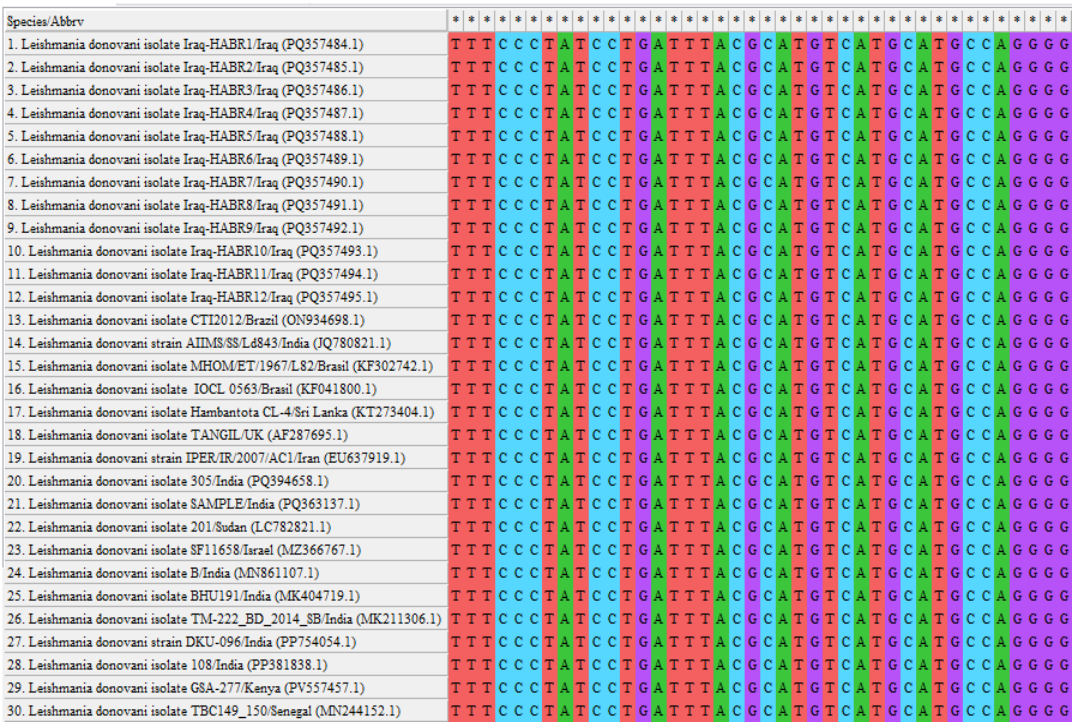


Figure 1. Total results of molecular PCR assay among totally 285 individuals.

Table 1. Homology sequence identity (%) of the current study and global NCBI-GenBank *L. donovani* isolates.

Local isolate		NCBI-BLAST isolate				Identity
Name	Access. No.	Isolate	Source	Country	Access. No.	(%)
Iraq-HABR1	PQ357484.1	CTI2012	Dog	Brazil	ON934698.1	99.92
Iraq-HABR2	PQ357485.1	CTI2012	Dog	Brazil	ON934698.1	99.30
Iraq-HABR3	PQ357486.1	CTI2012	Dog	Brazil	ON934698.1	98.93
Iraq-HABR4	PQ357487.1	CTI2012	Dog	Brazil	ON934698.1	98.65
Iraq-HABR5	PQ357488.1	CTI2012	Dog	Brazil	ON934698.1	99.94
Iraq-HABR6	PQ357489.1	CTI2012	Dog	Brazil	ON934698.1	99.92
Iraq-HABR7	PQ357490.1	CTI2012	Dog	Brazil	ON934698.1	99.90
Iraq-HABR8	PQ357491.1	CTI2012	Dog	Brazil	ON934698.1	98.50
Iraq-HABR9	PQ357492.1	CTI2012	Dog	Brazil	ON934698.1	99.83
Iraq-HABR10	PQ357493.1	CTI2012	Dog	Brazil	ON934698.1	98.95
Iraq-HABR11	PQ357494.1	CTI2012	Dog	Brazil	ON934698.1	99.15
Iraq-HABR12	PQ357495.1	CTI2012	Dog	Brazil	ON934698.1	99.82



Species/Abbrv	*	*	*	*	*								*	*								*				*			*				*		
1. Leishmania donovani isolate Iraq-HABR1/Iraq (PQ357484.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
2. Leishmania donovani isolate Iraq-HABR2/Iraq (PQ357485.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
3. Leishmania donovani isolate Iraq-HABR3/Iraq (PQ357486.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
4. Leishmania donovani isolate Iraq-HABR4/Iraq (PQ357487.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
5. Leishmania donovani isolate Iraq-HABR5/Iraq (PQ357488.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
6. Leishmania donovani isolate Iraq-HABR6/Iraq (PQ357489.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
7. Leishmania donovani isolate Iraq-HABR7/Iraq (PQ357490.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
8. Leishmania donovani isolate Iraq-HABR8/Iraq (PQ357491.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
9. Leishmania donovani isolate Iraq-HABR9/Iraq (PQ357492.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
10. Leishmania donovani isolate Iraq-HABR10/Iraq (PQ357493.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
11. Leishmania donovani isolate Iraq-HABR11/Iraq (PQ357494.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
12. Leishmania donovani isolate Iraq-HABR12/Iraq (PQ357495.1)	T	A	T	C	G	G	C	T	T	G	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T
13. Leishmania donovani isolate CTI2012/Brazil (ON934698.1)	T	A	T	C	G	G	C	T	T	T	G	T	T	G	G	T	T	T	T	A	A	A	G	G	T	C	T	A	T	T	G	G	A	G	A	T	T	
14. Leishmania donovani strain AIIMS/SS/Ld843/India (JQ780821.1)	T	A	T	C	G	T	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
15. Leishmania donovani isolate MHOM/ET/1967/L82/Brasil (KF302742.1)	T	A	T	C	G	G	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
16. Leishmania donovani isolate IOCL 0563/Brasil (KF041800.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
17. Leishmania donovani isolate Hambantota CL-4/Sri Lanka (KT273404.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
18. Leishmania donovani isolate TANGIL/UK (AF287695.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
19. Leishmania donovani strain IPER/IR/2007/AC1/Iran (EU637919.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
20. Leishmania donovani isolate 305/India (PQ394658.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
21. Leishmania donovani isolate SAMPLE/India (PQ363137.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
22. Leishmania donovani isolate 201/Sudan (LC782821.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
23. Leishmania donovani isolate SF11658/Israel (MZ366767.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
24. Leishmania donovani isolate B/India (MN861107.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
25. Leishmania donovani isolate BHU191/India (MK404719.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
26. Leishmania donovani isolate TM-222_BD_2014_SB/India (MK211306.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
27. Leishmania donovani strain DKU-096/India (PP754054.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
28. Leishmania donovani isolate 108/India (PP381838.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
29. Leishmania donovani isolate G8A-277/Kenya (PV557457.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A
30. Leishmania donovani isolate TBC149_150/Senegal (MN244152.1)	T	A	T	C	G	A	T	T	G	T	A	C	T	T	G	A	G	A	T	T	T	T	A	C	A	G	C	T	T	C	T	A	A	A	T	A	T	A

Figure 2. MSA for the current study and global NCBI-GenBank *L. donovani* isolates using the MEGA-11 software.

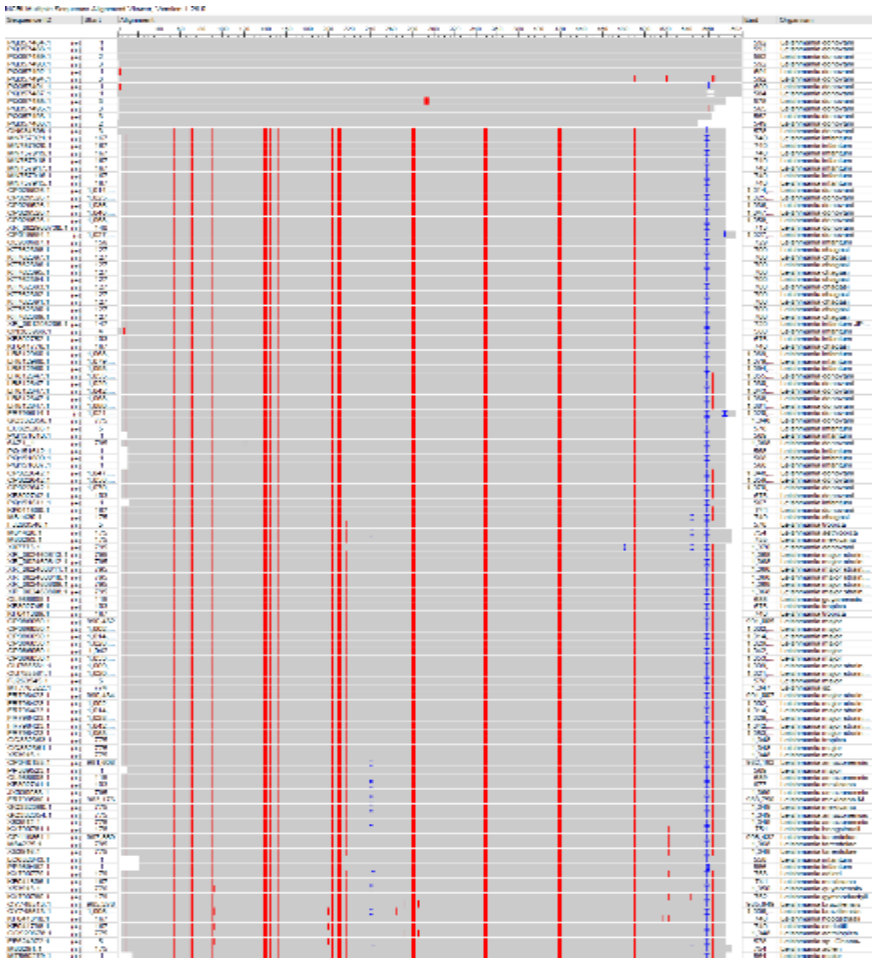


Figure 3. MSA showing nucleic acid differences between the study *L. donovani* isolates and the global NCBI-GenBank *L. donovani* isolates/strains using the NCBI-Viewer.

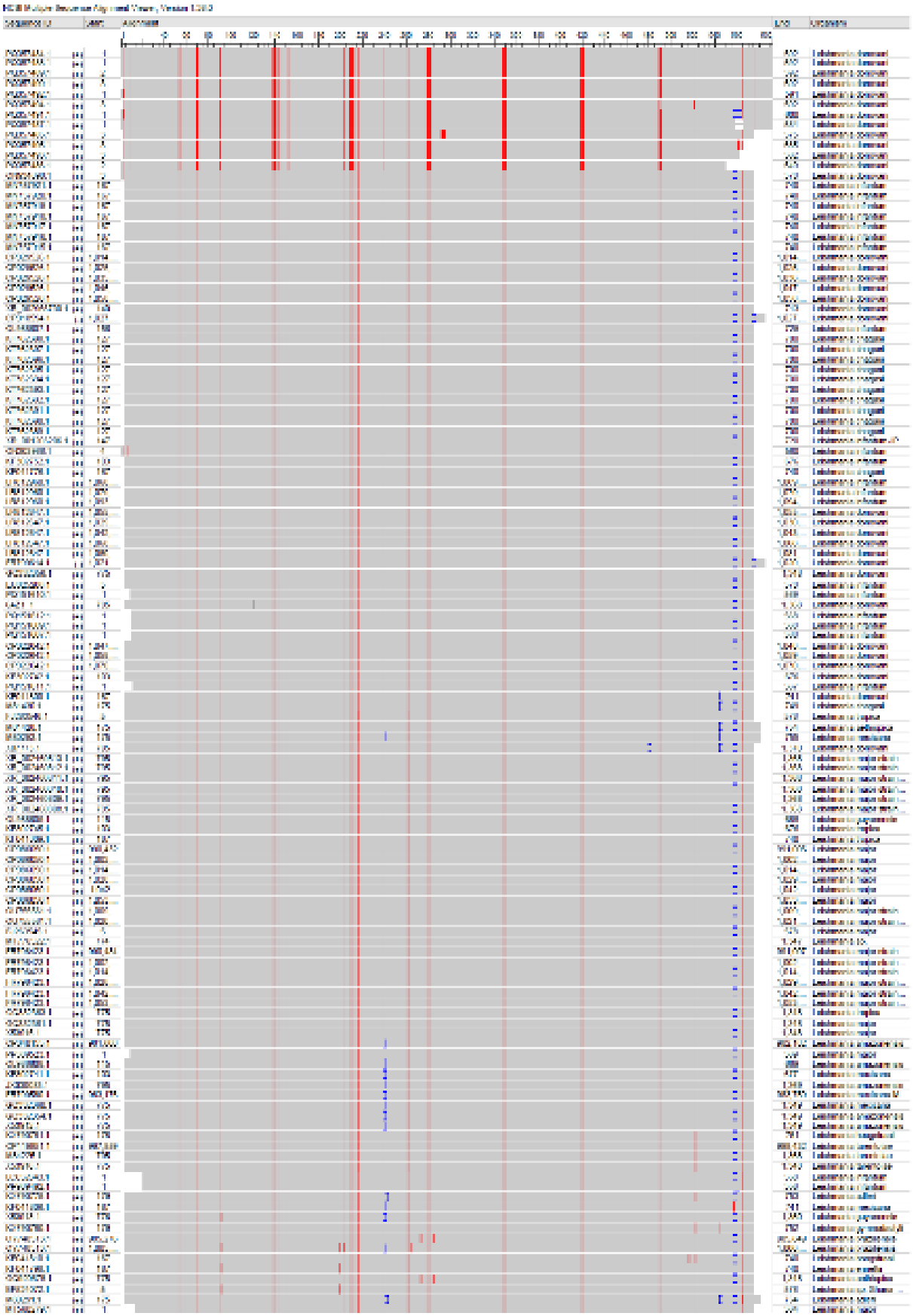


Figure 4. MSA showing nucleic acid similarity between the study *L. donovani* isolates and the global NCBI-GenBank *L. donovani* isolates/strains using the NCBI-Viewer.

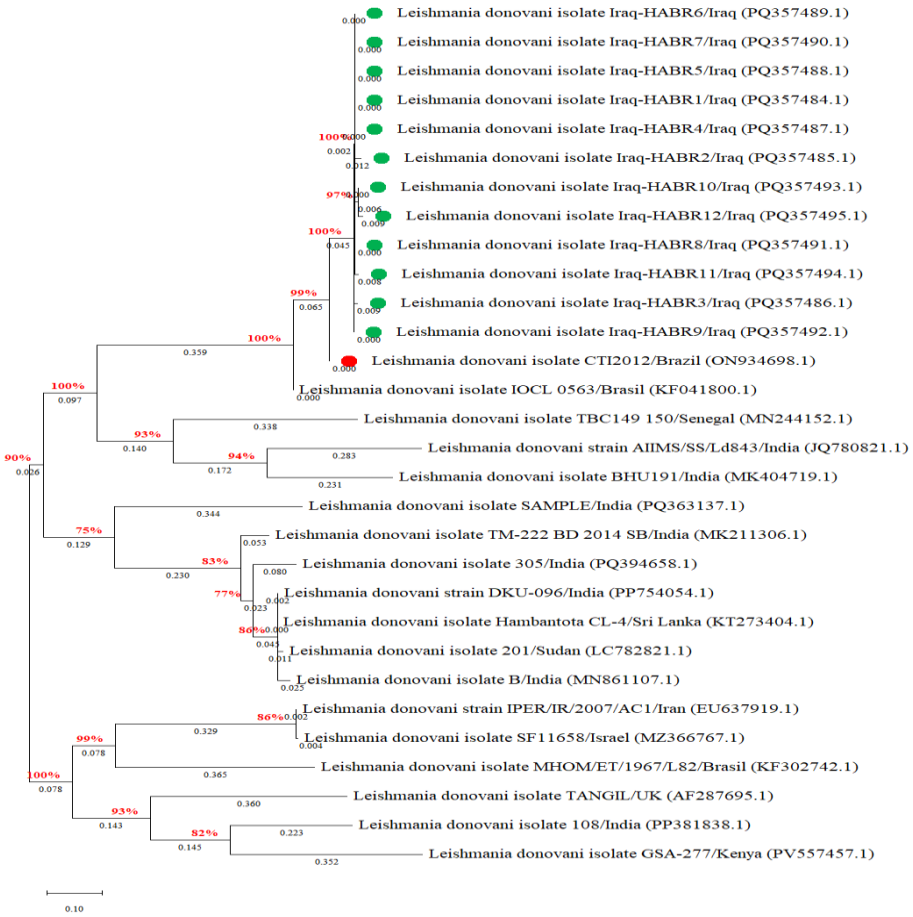


Figure 5. Phylogenetic tree analysis of the current study and global NCBI-GenBank *L. donovani* isolates.

Immunology

The quantitative measurement of IL-1⊚, IL-10, IFN-⊚, and TNF-⊚ levels was shown a marked variation between the negatively and positively infected groups of study population. Significantly, IL-1⊚ was reduced ($p<0.0001$; 95%CI: 17.02 to 38.15) in positively infected *L. donovani* individuals (18.4 ± 0.57 pg/ml) when compared to those of negatively infection (35.17 ± 1.44 pg/ml), (Figure 6). Concerning IL-10, values of positively infected individuals (58.77 ± 3.69 pg/ml) were significantly ($p<0.0009$; 95%CI: 19.88 to 67.81) higher than reported in negatively infected individuals (24.82 ± 2.38 pg / ml), see Figure 7.

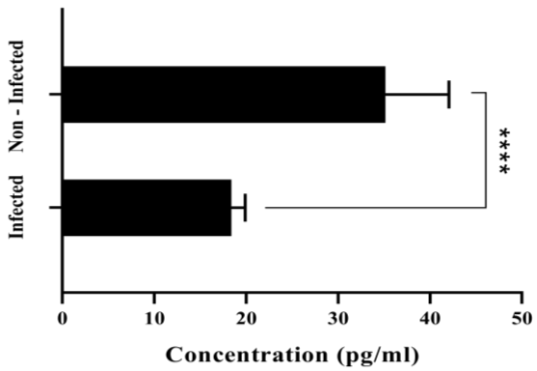


Figure 6. Concentration of IL-1⊚ in negatively and positively infected groups of study population (Total No: 285).

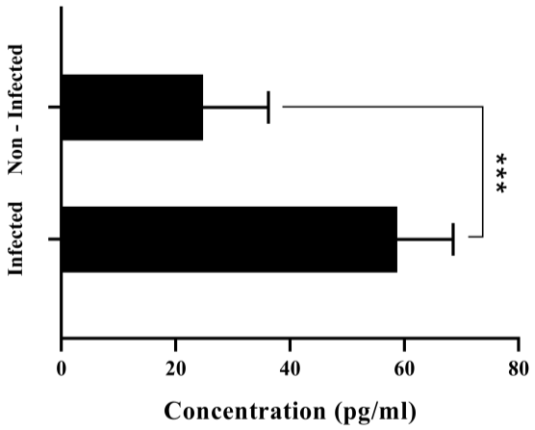


Figure 7. Concentration of IL-10 in negatively and positively infected groups of study population (Total No: 285).

Regarding IFN-◎, significant elevation (p<0.012; 95%CI: 18.49 to 59.03) was seen in positively infected *L. donovani* individuals (48.31 ± 4.38 pg/ml) in comparison with those of negatively infections (24.76 ± 3.02 pg/ml), see Figure 8.

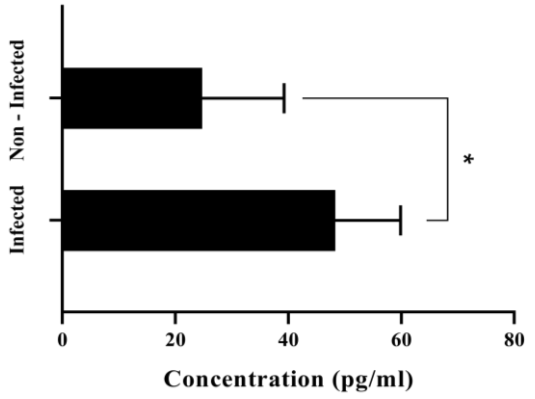


Figure 8. Concentration of IFN-◎ in negatively and positively infected groups of study population (Total No: 285).

Values of TNF-◎ were elevated significantly (p<0.023; 95%CI: 55.20 to 197.5) was seen in positively infected *L. donovani* individuals (180.33 ± 6.69 pg/ml) in comparison with those of negatively infections (66.75 ± 5.58 pg/ml), see Figure 8.

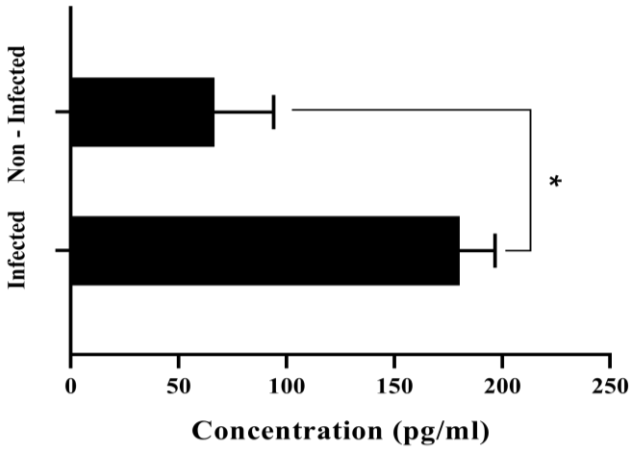


Figure 9. Concentration of TNF-◎ in negatively and positively infected groups of study population (Total No: 285).

Discussion

Since 1991, visceral leishmaniasis has extended to new areas affected before rarely in Iraq such as the southern governorates, and the risk of outbreaks was higher after 2003 (Jassim et al., 2006). The findings of this study identified that 4.21% of study population were positively infected with *L. donovani* isolates. Visceral leishmaniasis caused by L. infantum and *L. donovani* remains one of the most neglected endemic diseases in Iraq due to the low number of conducted studies (Taj-el Deen and Al Alousi, 1954; Niazi, 1980; Sukkar, 1984; Jassim et al., 2006; Hashim et al., 2007; Al-Ani et al., 2012; Rahi et al., 2013; Yaseen and Ali, 2016; Al-Hussaini et al., 2017; Mohammed et al., 2019; Yaseen and Ali, 2019; Al-Taei et al., 2022; Jarallah et al., 2022; Alyasiri and Ali, 2024; Dakeel and Mohsein, 2024; Hamoodi et al., 2025); nonetheless, no available data about the occurrence of visceral leishmaniasis in clinically asymptomatic individuals in addition to complete absence of phylogenetic information among all these studies. However, our molecular findings were in agreement with Al-Ani et al. (2012) who reported that the positive cases among 1231 clinically suspected patients admitted to Al-Ramadi Maternity and Children's Hospital was 32 (2.6%) throughout the bone marrow examination and IFAT. Another survey study in Karbala governorate has been detected that the infection rate of visceral leishmaniasis caused by *L. donovani* was 6.75% using culture (Al-Taei et al., 2022). Dakeel and Mohsein (2024) who identified that the occurrence of *L. donovani* suspected cases in Diwaniyah governorate by the ICT was 6.25% with confirming the positive infection using the culture, IFAT and PCR. In comparison to worldwide studies, the prevalence rate of *L. donovani* using the molecular tools was 9-25% in Brazilian blood donors (Luz et al., 1997), 0.44-3.83% in Sudan (Khalil et al., 2002), 9% (5-15% per cluster) in Nepal (Rijal et al., 2010), 0.26-7.26% in India (Chapman et al., 2018), and 0.9-15.8% in Ethiopia (Belay et al., 2025). This variation in incidence rate of visceral leishmaniasis caused by *L. donovani* might be attributed to the diagnostic methods, clinical forms of infection, and seasonal variations.

Phylogenetically, the findings of the presents study demonstrated that the local *L. donovani* isolates were close-related to the Brazilian *L. donovani* isolated from dogs. In recent epidemiological researches, there is strong evidence implicates canids particularly domestic dogs as a significant reservoirs in the transmission cycle of *L. donovani* to humans, especially in areas where canine visceral leishmaniasis is endemic (Quinnell and Courtenay, 2009; Campino and Maia, 2018; Beasley et al., 2021). Therefore, understanding the dynamic of Leishmania infection in canine population including prevalence, clinical presentation, and parasite load is critical for elucidating their role in maintaining the parasite's life cycle and facilitating spillover events to human populations (Courtenay et al., 2017; Cosma et al., 2024; Silva-Moreira et al., 2025).

The results of immune markers measured in current study indicate a significant reduction of IL-1◎ and marked increases in values of IL-10, TNF-◎, and TNF-◎ in cases of positively *L. donovani* infections. These results were in agreement with that recorded by several researchers (Bersudsky et al., 2000; Gupta et al., 2017; Kariyawasam et al., 2017). Elsewhere, human and mouse models have shown that resistance to infection by lenticous leishmaniasis and self-healing are related to the acquisition of a robust Th1 response with IL-1 2, IFN-◎ and TNF-◎ expression and vulnerability and persistence with the parasite are correlates of a predominant Th2 response, with IL-4, IL-5 and IL-6 (Tripathi et al., 2007; Hartley et al., 2013; Maspi et al., 2016; Moafi et al., 2017). Reiner et al. (1990) found in vitro that the production of IL-1 in infected-peripheral blood monocytes with Leishmania was diminished probably reflected the reduced translation of IL-1◎, and prepared cells to produce both IL-1 and TNF-◎ when further stimulated by Leishmania suggesting that *L. donovani* has acquired the ability to infect mononuclear phagocytes, without inducing the generation of two potentially protective monokines. Additionally, the effect of IFN-◎ in priming monocytes to secrete TNF-◎ in response to Leishmania infection and inhibit the effects of the IL-1 production could also have immunotherapy implications in the use of this lymphokine. Ghalib et al. (1993) established that IL-10 mRNA expression in lymphnodes removed during acute infection with *L. donovani* suggesting that production of IL-10 may play a role in regulating the immune responsiveness to visceral leishmaniasis. Matte and Descoteaux (2010) have explored the effect of infection by the amastigote-stage of *L. donovani* on IFN-◎ responses and pathways in mouse bone marrow-derived macrophages; and concluded an evidence of a novel action of *L.*

donovani amastigotes to disrupt the functions of IFN-◎ treated macrophages; and a better insight into the strategies employed by this parasite to enable its survival within a cell. The immune response to *L. donovani* infection is highly complex, with macrophages serving as both the primary host cell for parasite, replication and crucial effector cells in parasite elimination (Saha et al., 2018; Yin et al., 2024). A critical component of this host response involves IL-1◎ that act as a potent pro-inflammatory mediator known for its pivotal role in orchestrating innate and adaptive immunity against various pathogens (Saha et al., 2018; Dayakar et al., 2019). This interleukin has multifaceted involvement in immunopathogenesis of *L. donovani* infection, and contributes to host resistance and susceptibility since it modulates macrophage function, influence T-cell differentiation and interacts with other immune pathways to shape outcome of infection (Dubie and Mohammed, 2020). However, the intricate host-parasite interactions within macrophage phagolysosome dictate disease progression, with remarkable adaptability of the parasite demonstrating to nutrient-limited or nutrient-rich intracellular environments (Séguin and Descoteaux, 2016; Carneiro and Peters, 2021; Bodhale et al., 2024).

Conclusion

This represents the first molecular study in Wasit province, and the first phylogenetic one in Iraq demonstrating that there was a close-relationship betwee the study *L. donovani* isolates and the global NCBI-GenBank isolates, and estimating levels of some immune markers in infected cases. Author suggests the great necessity of additional molecular phylogenetic and immune studies in cases of leishmaniasis. The zoonotic nature of *L. donovani* transmission especially the involvement of dogs, necessitate a thorough examination of canine reservoirs to inform targeted public health interventions.

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REFERENCES

Abbas, F., El Kossi, M., Kim, J. J., Shaheen, I. S., Sharma, A., Pararajasingam, R., and Halawa, A. (2019). Parasitic infestation in organ transplant recipients: A comprehensive review in the absence of robust evidence. Journal of The Egyptian Society of Nephrology and Transplantation, 19(2), 31-61.

Abbasi, E. (2025). Advancing insights into visceral leishmaniasis: challenges, innovations, and future directions in global disease management. Innovations, and future directions in global disease management.

Ajaj, E.A., Mohammad, H.A., and Gharban, H.A. (2021). First molecular confirmation of Coenurus cerebralis in sheep and goats with neurological behaviors in Iraq. Veterinary World, 14(6), 1420.

Al-Ani, Z.R., Al-Hamwandi, A.M., Al-Ma'aeeni, A.A., and Al-Ta'aie, M.K. (2012). Kala-azar in al-anbar governorate, western iraq. Anb Med J, 10(1), 41-49.

Al-Ardi, M.H. (2022). The geographical distribution patterns of human Leishmania species detected within molecular methods in Iraq: a systematic review and meta-analysis. Al-Kufa University Journal for Biology, 14(1), 1-15.

Al-Hayali, H.L., and Al-Kattan, M.M. (2021). Overview on epidemiology of leishmaniasis in Iraq. Rafidain journal of science, 30(1), 28-37.

Al-Hussaini, R.M.A., Al-Tufaili, R.N., and Hussein, R.A. (2017). Molecular study of pediatric visceral leishmaniasis in Mid-Euphrates Area, Iraq. International Journal of Science and Engineering Research, 8(8), 148-152.

Al-Joary, Y.I.M.A., and Al-Hamdani, M.A.M. (2024). First Molecular Identification of Phlebotomine Sandflies (Diptera: Psychodidae) in Nineveh Governorate, Northern of Iraq. Biomedical and Biotechnology Research Journal (BBRJ), 8(2), 187-193.

Al-Qahtani, A.A., Alhamlan, F.S., and Al-Qahtani, A.A. (2024). Pro-inflammatory and anti-inflammatory interleukins in infectious diseases: A comprehensive review. Tropical medicine and infectious disease, 9(1), 13.

Al-Taei, H.T.A.A., Al-Qazwini, Y. M., and Zwair, H. (2022). A survey study for prevalence of visceral leishmaniasis in Karbala, Iraq. International journal of health sciences, 6(S6), 9385-9396.

Alyasiri, A.J., and Ali, H.H. (2024). A clinicoepidemiological study on kala-zar disease from areas infested with the *Leishmania donovani* in Al-Muthanna province, Iraq. Journal of Applied Health Sciences and Medicine, 4(2), 14-28.

Beasley, E.A., Pessôa-Pereira, D., Scorza, B.M., and Petersen, C.A. (2021). Epidemiologic, clinical and immunological consequences of co-infections during canine leishmaniosis. Animals, 11(11), 3206.

Belay, H., Abera, A., Aklilu, E., Bishaw, T., Alemu, A., Tasew, G., and Erko, B. (2025). Prevalence of asymptomatic *Leishmania donovani* infection and associated factors in Ethiopia: a systematic review and meta-analysis. BMC infectious diseases, 25(1), 78.

Bersudsky, M., Apte, R.N., and El-On, J. (2000). Interleukin 1α activity of peritoneal and bone marrow macrophages infected with Leishmania major and *Leishmania donovani* in vitro. Experimental parasitology, 94(3), 150-157.

Bodhale, N., Saha, S., Gurjar, D., Grandchamp, N., Sarkar, A., and Saha, B. (2024). *Leishmania donovani* mitogen-activated protein kinases as a host-parasite interaction interface. Cytokine, 179, 156627.

Campino, L., and Maia, C. (2018). The role of reservoirs: canine leishmaniasis. Drug Resistance in Leishmania Parasites: Consequences, Molecular Mechanisms and Possible Treatments, 59-83.

Carneiro, M.B., and Peters, N.C. (2021). The paradox of a phagosomal lifestyle: how innate host cell-Leishmania amazonensis interactions lead to a progressive chronic disease. Frontiers in immunology, 12, 728848.

Chapman, L.A., Morgan, A.L., Adams, E.R., Bern, C., Medley, G.F., and Hollingsworth, T.D. (2018). Age trends in asymptomatic and symptomatic *Leishmania donovani* infection in the Indian subcontinent: A review and analysis of data from diagnostic and epidemiological studies. PLoS neglected tropical diseases, 12(12), e0006803.

Cosma, C., Maia, C., Khan, N., Infantino, M., and Del Riccio, M. (2024). Leishmaniasis in humans and animals: A One Health approach for surveillance, prevention and control in a changing world. Tropical Medicine and Infectious Disease, 9(11), 258.

Courtenay, O., Peters, N.C., Rogers, M.E., and Bern, C. (2017). Combining epidemiology with basic biology of sand flies, parasites, and hosts to inform leishmaniasis transmission dynamics and control. PLoS pathogens, 13(10), e1006571.

Dakeel, M.H., and Mohsein, A.B. (2024). Epidemiological and molecular study of the *Leishmania donovani* . Azerbaijan Pharmaceutical and Pharmacotherapy Journal, 23(2), 157-165.

Dayakar, A., Chandrasekaran, S., Kuchipudi, S.V., and Kalangi, S. K. (2019). Cytokines: key determinants of resistance or disease progression in visceral leishmaniasis: opportunities for novel diagnostics and immunotherapy. Frontiers in immunology, 10, 670.

Dubie, T., and Mohammed, Y. (2020). Review on the role of host immune response in protection and immunopathogenesis during cutaneous leishmaniasis infection. Journal of immunology research, 2020(1), 2496713.

Ghalib, H.W., Piuvezam, M.R., Skeiky, Y.A., Siddig, M., Hashim, F.A., El-Hassan, A.M., and Reed, S.G. (1993). Interleukin 10 production correlates with pathology in human *Leishmania donovani* infections. The Journal of clinical investigation, 92(1), 324-329.

Gharban, H.A., Sray, A.H., and Essa, I.M. (2024). Serological prevalence of anti-Fasciola hepatica antibodies in sheep. Egyptian Journal of Veterinary Sciences, 55(6), 1583-1590.

Gow, I., Smith, N.C., Stark, D., and Ellis, J. (2022). Laboratory diagnostics for human Leishmania infections: a polymerase chain reaction-focussed review of detection and identification methods. Parasites and Vectors, 15(1), 412.

Gupta, A.K., Ghosh, K., Palit, S., Barua, J., Das, P.K., and Ukil, A. (2017). *Leishmania donovani* inhibits inflammasome-dependent macrophage activation by exploiting the negative regulatory proteins A20 and UCP2. The FASEB Journal, 31(11), 5087-5101.

Hamoodi, H.F., Aldeen, M.B., Aliwi, N.M., and Abdulkhaliq, S.H. (2025). The Prevalence of Some Epidemic Leishmania Spp. Isolated from Human Blood and Skin Samples. Al-Nahrain Journal of Science, 28(2), 106-112.

Hartley, M.A., Kohl, K., Ronet, C., and Fasel, N. (2013). The therapeutic potential of immune cross-talk in leishmaniasis. Clinical Microbiology and Infection, 19(2), 119-130.

Hashim, J.M., Galil, T.A., and Abdul-Kadim, S.H. (2007). Epidemiological and clinical study of visceral leishmaniasis in Najaf and Karbala Governorates. Karbala Journal of Medicine, 1, 124-130.

Jaffar, Z.A., Jasim, A.M., and Majeed, G.H. (2024). Detection of Visceral Leishmaniasis by Leishmania infantum using kDNA. Magazine of Al-Kufa University for Biology, 16(2).

Jarallah, H.M., Aabadi, H.I., and Shaalan, N.N. (2022). Environmental risk factors associated with distribution of visceral leishmaniasis in Marshlands of Iraq. In AIP Conference Proceedings. AIP Publishing LLC. 2386(1), 020031).

Jassim, A.K., Maktoof, R., Ali, H., Bodosan, B., and Campbell, K. (2006). Report: visceral leishmaniasis control in Thi Qar Governorate, Iraq, 2003. Eastern Mediterranean Health Journal, 12 (Supp. 2), S230-S237.

Kariyawasam, R., Grewal, J., Lau, R., Purssell, A., Valencia, B.M., Llanos-Cuentas, A., and Boggild, A.K. (2017). Influence of leishmania RNA virus 1 on proinflammatory biomarker expression in a human macrophage model of American tegumentary leishmaniasis. The Journal of Infectious Diseases, 216(7), 877-886.

Khalil, E.A.G., Zijlstra, E.E., Kager, P.A., and El Hassan, A.M. (2002). Epidemiology and clinical manifestations of *Leishmania donovani* infection in two villages in an endemic area in eastern Sudan. Tropical Medicine and International Health, 7(1), 35-44.

Kumar, A., Pandey, S.C., and Samant, M. (2020). A spotlight on the diagnostic methods of a fatal disease Visceral Leishmaniasis. Parasite immunology, 42(10), e12727.

Luz, K.G., Da Silva, V.O., Gomes, E.M., Machado, F.C., Araujo, M.F., Fonseca, H.E., and Palatnik-de Sousa, C.B. (1997). Prevalence of anti-*Leishmania donovani* antibody among Brazilian blood donors and multiply transfused hemodialysis patients. The American journal of tropical medicine and hygiene, 57(2), 168-171.

Maspi, N., Abdoli, A., and Ghaffarifar, F. (2016). Pro-and anti-inflammatory cytokines in cutaneous leishmaniasis: a review. Pathogens and global health, 110(6), 247-260.

Matte, C., and Descoteaux, A. (2010). *Leishmania donovani* amastigotes impair gamma interferon-induced STAT1α nuclear translocation by blocking the interaction between STAT1α and importin-α5. Infection and immunity, 78(9), 3736-3743.

Mehrotra, S., Tiwari, R., Kumar, R., and Sundar, S. (2025). Advances and Challenges in the Diagnosis of Leishmaniasis. Molecular Diagnosis and Therapy, 29(2), 195-212.

Moafi, M., Rezvan, H., Sherkat, R., Taleban, R., Asilian, A., Hamid Zarkesh-Esfahani, S., and Ansari, N. (2017). Comparison of pro-inflammatory cytokines of non-healing and healing cutaneous leishmaniasis. Scandinavian journal of immunology, 85(4), 291-299.

Mohammed, N.S., Muhsen, S.S., Husain, S.K., and Sugaier, A.R.N. (2019). The sequence of GP63 gene of Leishmania dononvani isolated from visceral leishmaniasis patients in Diyala province. Biochemical and Cellular Archives, 19(1).

Niazi, A.D. (1980). Studies in epidemiology and seroepidemiology of visceral leishmaniasis in Iraq Doctoral dissertation, University of London, Faculty of Medicine, London School of Hygiene and Tropical Medicine.

Obeagu, E.I. (2024). Role of cytokines in immunomodulation during malaria clearance. Annals of Medicine and Surgery, 86(5), 2873-2882.

Perez, R.L., Chase, J., and Tanner, R. (2024). Shared challenges to the control of complex intracellular neglected pathogens. Frontiers in Public Health, 12, 1423420.

Quinnell, R.J., and Courtenay, O. (2009). Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. Parasitology, 136(14), 1915-1934.

Rahi, A.A., Ali, M.A., Valian, H.K., Mohebali, M., and Khamesipour, A. (2013). Seroepidemiological studies of visceral leishmaniasis in Iraq. Sch. J. App. Med. Sci, 1(6), 985-989.

Reimão, J.Q., Coser, E.M., Lee, M.R., and Coelho, A.C. (2020). Laboratory diagnosis of cutaneous and visceral leishmaniasis: current and future methods. Microorganisms, 8(11), 1632.

Reiner, N.E., Ng, W., Wilson, C.B., McMaster, W.R., and Burchett, S.K. (1990). Modulation of in vitro monocyte cytokine responses to *Leishmania donovani* . Interferon-gamma prevents parasite-induced inhibition of interleukin 1 production and primes monocytes to respond to Leishmania by producing both tumor necrosis factor-alpha and interleukin 1. The Journal of clinical investigation, 85(6), 1914-1924.

Rijal, S., Uranw, S., Chappuis, F., Picado, A., Khanal, B., Paudel, I. S., and Boelaert, M. (2010). Epidemiology of *Leishmania donovani* infection in high-transmission foci in Nepal. Tropical Medicine and International Health, 15, 21-28.

Saha, A., Basu, M., and Ukil, A. (2018). Recent advances in understanding *Leishmania donovani* infection: The importance of diverse host regulatory pathways. IUBMB life, 70(7), 593-601.

Saha, A., Roy, S., and Ukil, A. (2022). Cytokines and signaling networks regulating disease outcomes in leishmaniasis. Infection and Immunity, 90(8), e00248-22.

Séguin, O., and Descoteaux, A. (2016). Leishmania, the phagosome, and host responses: The journey of a parasite. Cellular immunology, 309, 1-6.

Silva-Moreira, A.L., Serravite, A.M., Rios-Barros, L.V., de Menezes, J.P.B., Horta, M.F., and Castro-Gomes, T. (2025). New insights into the life cycle, host cell tropism, and infection amplification of Leishmania spp. Infection and Immunity, e00123-25.

Sukkar, F. (1986). Evaluation of visceral leishmaniasis control programme. Bulletin of Endemic Diseases, 27(1-4), 63-76.

Taj-el Deen, S.D., and Al Alousi, K. (1954). Kala-Azar in Iraq: Report of Four Cases. Journal of the Faculty of Medicine, 18(1/2), 15-19.

Tao, J., and Jia, W. (2024). Leishmania. In Molecular Medical Microbiology (pp. 3061-3068). Academic Press.

Tom, A., Kumar, N.P., Kumar, A., and Saini, P. (2024). Interactions between Leishmania parasite and sandfly: a review. Parasitology Research, 123(1), 6.

Tripathi, P., Singh, V., and Naik, S. (2007). Immune response to leishmania: paradox rather than paradigm. FEMS Immunology and Medical Microbiology, 51(2), 229-242.

Volpedo, G., Pacheco-Fernandez, T., Holcomb, E. A., Cipriano, N., Cox, B., and Satoskar, A. R. (2021). Mechanisms of immunopathogenesis in cutaneous leishmaniasis and post kala-azar dermal leishmaniasis (PKDL). Frontiers in cellular and infection microbiology, 11, 685296.

Wen, T. H., Tsai, K. W., Wu, Y. J., Liao, M. T., Lu, K. C., and Hu, W. C. (2021). The framework for human host immune responses to four types of parasitic infections and relevant key JAK/STAT signaling. International Journal of Molecular Sciences, 22(24), 13310.

Yaseen, F.T., and Ali, H.Z. (2016). Using of Species-Specific Primers for Molecular Diagnosis of in Vitro Promastigotes of *Leishmania donovani* . Iraqi Journal of Science, 824-829.

Yaseen, F.T., and Ali, H.Z. (2019). Identification of *Leishmania donovani* isolates by polymerase chain reaction. Iraqi journal of biotechnology, 18(2), 11-17.

Yasmin, H., Adhikary, A., Al-Ahdal, M.N., Roy, S., and Kishore, U. (2022). Host–pathogen interaction in leishmaniasis: immune response and vaccination strategies. Immuno, 2(1), 218-254.

Yin, S., Li, J., Chen, J., Zhou, Q., bin pei Duan, D., Lai, M., and Zheng, Z. (2024). LdCyPA attenuates MAPK pathway to assist *Leishmania donovani* immune escape in host cells. Acta Tropica, 251, 107114.